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Meiotic effects in chromosomally derived male sterility of mice

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Summary. Much sterility in male (though not in female) mice can be related to the presence of specific types of chromosome anomaly. These include the XXY and XYY conditions, failures of association of X and Y, heterozygosity for reciprocal translocations involving the X or Y chromosomes as well as some which involve autosomes only, tertiary trisomic derivatives of these and certain multiple Robertsonian translocations. In all of these the sterility stems from a defect of spermatogenesis, which leads to the production of few or no spermatozoa. Studies of reciprocal translocations are of particular value for elucidating the mechanisms involved because different ones exhibit an almost complete range of effects, from those with normal sperm production to those in which spermatogenesis stops at the onset of meiosis. Between these extremes a number of characteristic patterns can be discerned but in general the cessation of spermatogenesis tends to be graded rather than abrupt. The effects on spermatogenesis are associated with special characteristics of the translocations themselves, namely a tendency for one point of exchange to be close to (or even within) centromeric heterochromatin and the other fairly distal on the chromosomes involved. This frequently leads to the formation of long and short marker chromosomes and to the production of viable tertiary trisomic mice, which are also male-sterile as a rule.

Mammalian spermatogenesis is a complex process, which is influenced by a large number of both intrinsic and extrinsic factors. Only in recent years has it been realised to what extent the intrinsic control depends on the normal arrangement of the genome. Chromosome anomalies which have no other discernible effects in the individuals carrying them may nevertheless completely disrupt the process of spermatogenic maturation in the male. In this respect, the immature male germ-cell differs markedly from the immature female one, which seems little affected by chromosome anomalies. It also differs from the mature spermatozoon, which is capable of fertilization even when it carries gross chromosomal anomalies which would be lethal in a zygote.

Table 1 illustrates the range of chromosome anomalies which affect spermatogenesis in the mouse and shows that some of these act at a certain stage while others are less specific. The XXY and XYY conditions are very rare in mice. XXY mice appear to have no germ-cells, though Sertoli cells remain (Ford *et al.*, 1975). The same seems true of human XXY individuals, in which the spermatogenic activity described in some reports may be connected with XY/XXY mosaicism (Chandley,

1975). The XYY condition is associated with post-meiotic spermatogenic breakdown, but with some formation of spermatids and spermatozoa. Although sterility is usually complete, a male XYY mouse which was fertile when young has been reported (Evans, Beechey and Burtenshaw, 1978). XYY men are frequently fertile, although some attend clinics for the sub-fertile and are then frequently found to have spermatogenic impairment, which may be severe (Chandley, 1975). In X-Y dissociation at metaphase I, which is also found in both man (Chandley *et al.*, 1976) and mouse (Beechey, 1973) there appears to be a single critical stage of spermatogenic arrest : at the first meiotic division, so that few or no secondary spermatocytes are formed. Possibly related to this is the spontaneous univalence described by Purnell (1973) in a single male mouse with small testes in which very few spermatozoa were found. An average of only 7 bivalents per spermatocyte were found at metaphase I, although X and Y chromosomes were occasionally paired. Pearson *et al.* (1970) have described a similar condition in man.

TABLE 1

Some chromosomal anomalies which affect fertility in male mice

Type of anomaly	Nature of effect	References
XXY	No spermatogenic cells	Cattanach (1961), Slizynski (1964), Ford *et al.* (1975)
XYY	Variable degree of spermatogenic impairment, but some spermatids and spermatozoa usually formed	Cattanach and Pollard (1969), Rathenburg and Muller (1973), Evans *et al.* (1977)
X-Y separation	No secondary spermatocytes	Beechey (1973), Chandley *et al.* (1976)
X-autosome translocations	Usual pachytene arrest but T16H continues to metaphase I and II	Lyon *et al.* (1964), Russell and Montgomery (1969), Eicher (1970)
Y-autosome translocations	Spermiogenic impairment	Léonard and Deknudt (1969), Cacheiro *et al.* (1974)
Autosome-autosome translocations (some)	Variable degree of spermatogenic impairment	Lyon and Meredith (1966), Cacheiro *et al.* (1974), Searle (1974)
Tertiary trisomies derived from autosome-autosome translocations	As above	As above
Common arms in translocations	Variable degree of spermatogenic impairment ; some fertile	Carter *et al.* (1956), Evans (1976) Winking and Gropp (1977)
Multiple univalents	Spermiogenic impairment	Purnell (1973)

Translocations involving the X or Y chromosomes are well known to cause sterility in male mice, the effect on spermatogenesis tending to be more severe in those involving the X-chromosome. Combinations of translocations with a common chromosome also tend to be male-sterile (Carter, Lyon and Phillips, 1956) and may even be lethal when each involves the same two chromosomes (Beechey and Searle, 1975). Double heterozygotes for Robertsonian translocations with a common arm are often male-sterile (Evans, 1976), as in four different combinations involving chr 8

(Winking and Gropp, 1977). Spermatozoa are greatly reduced in number or are completely absent, while one example studied in detail revealed a breakdown in spermatid maturation setting in at stage IX-3 (Döring, Winking and Gropp, 1975). However, some combinations of the kind described are fully fertile while others are intermediate, i.e. with lowered fertility (Evans, 1976).

Double heterozygotes for reciprocal translocations may also be male-sterile (Carter, Lyon and Phillips, 1956) even when neither of the single heterozygotes are so. As Evans (1976) has pointed out, these double reciprocal or Robertsonian translocations commonly form long chain multivalent configurations at meiosis. It is interesting to note that the formation of chain multivalents, rather than rings, is also a very characteristic feature of male-sterile reciprocal translocations involving the autosomes, as discussed below.

Lyon and Meredith (1966) were the first to show that certain translocations which did not involve the sex-chromosomes nevertheless caused sterility of the male heterozygote because of spermatogenic impairment. They also noted that the translocations tended to give a high proportion of chain configurations at meiotic metaphase I, in contrast to the usual preponderance of rings. Further work has amply confirmed this tendency (Cacheiro, Russell and Swartout, 1974 ; Searle, 1974).

We have generated a number of translocations of this type, as well as X-autosome translocations, in an experiment in which male mice were given doses of 0-1200 rad

TABLE 2

Spermatogenic anomalies and testis weights in heterozygotes for male-sterile translocations (T) and tertiary trisomics (Ts). MI = metaphase-I

Translocation	Anomalies	Testis weight (p. 100 of normal)
T(5 ; 12) 31 H	Reduced numbers of spermatozoa	58
Ts 31 H *	Few spermatids, practically no sperm	31
T(6 ; 12) 32 H	Few sperm, spermatids also reduced	53
T(4 ; 8) 36 H	Many MI spermatocytes but few later stages	44
T(X ; 11) 38 H	Primary spermatocytes, not reaching MI	34
Ts 38 H *	MI reached but no sperm	?
T(X ; 4) 37 H	Spermatocytes mainly stop at pachytene but a few reach MI	32
T(16 ; 17) 43 H	Very few post-meiotic stages	32
T(1 ; 7) 40 H	Spermatogonia and early spermatocytes only	27
T(11 ; 19) 42 H	Primary spermatocytes reach pachytene only	26

* With the normal balanced chromosome set (20 II) plus the small translocation product.

acute X-irradiation (Searle *et al.*, 1974). Daughters were tested for semi-sterility and were bred from if they produced sterile sons. Another male-sterile translocation (T31H) in the present series was induced by irradiation of female mice (Searle and Beechey, 1974). Table 2 lists these translocations, as well as two tertiary trisomic conditions derived from them which were also male-sterile, and gives approximate testis weights, mainly based on measurements at 6-8 weeks of age, as percentages of normal. Information on the chromosomes involved (placed in parentheses within the translocation name) was obtained by the ASG and trypsin banding methods, with Giemsa staining.

Table 2 shows that in general there was quite a good correlation between testis weight reduction and the severity of spermatogenic effects, which ranged from a reduced sperm-count in T31H to the presence of only the earliest meiotic stages in T40H and T42H. Detailed quantitative studies of the spermatogenic population in seminiferous tubules have not yet been made, but examination of air-dried preparations made from suspensions of germ-cell (Evans, Breckon and Ford, 1964) as well as histological sections of whole testes made it clear that the effect tended to be graded rather than abrupt, with some germ-cells surmounting a particular critical stage but failing to pass the next one. Thus in T37H most spermatocytes failed to progress further than pachytene, but a few managed to reach metaphase I. There seemed to be critical stages at pachytene, around meiotic metaphase I and during sperm formation, but more work is needed to confirm observations. Cacheiro, Russell and Swartout (1974) reported that in their series of 42 sterile males resulting from post-meiotic treatment with mutagens, 15 showed a spermatogenic block in pachytene and 9 at diakinesis, while 17 had some mature spermatids. One lacked spermatogenic cells altogether, but seemed to be chromosomally normal. In our series, there were few signs of any spermatogenic wave in seminiferous tubules from heterozygotes for translocations with the greatest effect, but there was heterogeneity with respect to severity of effect between different segments of tubule. Some sections of tubule looked remarkably empty, with considerable depletion in the numbers of early spermatocyte stages. Large darkly-staining cells were found in the lumen of tubules and sometimes these looked multinucleate. In general, there was no marked difference between the balanced translocation carriers and the tertiary trisomics in the types of effect seen, but there was a difference in the severity of effect.

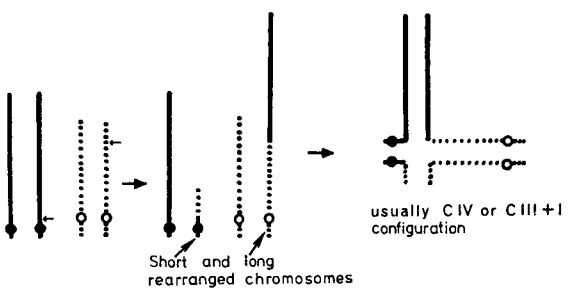

FIG. 1. — *Generation and pachytene configuration of typical male-sterile translocation, with positions of break-points marked with small arrows.*

The preponderant formation by these male-sterile translocation heterozygotes of chain configurations (CIV or CIII + I) at metaphase I (if this is reached) results from failure of chiasma formation in one arm or in adjacent arms of the translocation cross (fig. 1). This is associated with formation of particularly long and/or short translocation products, i.e. somatic marker chromosomes like the well-known T6 marker which is itself male-sterile on certain genetic backgrounds (Baranov and Dyban, 1968). Six out of the eight male-sterile translocations described here were somatic markers of this type. Of the remaining two, T36H included a submetacentric marker, while T43H had a break-point close to the centromere of Chr 16, with translocation of C-band material (centromeric heterochromatin) to Chr 17, thus generating a marker chromosome in C-banded preparations. As figure 1 shows, the characteristics of these translocations are those expected if one break-point is near the centromere and the other fairly distal on the chromosome (Searle, 1974). Figure 2 gives the approximate break-point positions for the Harwell series of male-sterile translocations to show that this is indeed the case. Since at meiotic metaphase in female carriers the short translocation marker may frequently segregate independently because of failure of chiasma formation, this type of translocation is likely to generate tertiary trisomic and monosomic zygotes, which may be viable because of the relatively small amount of chromosomal material which is duplicated or deficient.

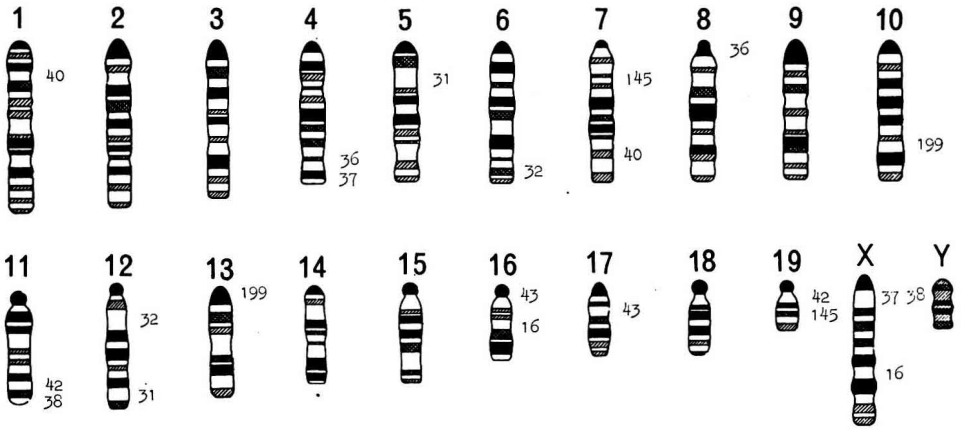

FIG. 2. — *Approximate positions of break-points in Harwell series of male-sterile translocations, in relation to the chromosome banding pattern in the mouse* (Nesbitt and Francke, 1973). For further details refer to Beechey *et al.*, 1976 (*T31H*) ; Dev *et al.*, 1974 (*T199H*) ; Eicher and Washburn, 1977 (*T16H* and *T145H*) ; Evans, Beechey and Searle, 1977 (others).

There can be little doubt these translocations act autonomously on the germ-cells carrying them, even though they may have systemic effects of which we are not yet aware. In males, this type of translocation has only been recovered from post-meiotic irradiation, in which the treated germ-cell does not divide further before forming the zygote. Presumably, these translocations are not recovered after spermatogonial irradiation because the germ-cells carrying them are eliminated during subsequent spermatogenesis, as a result of autonomous action. This phenomenon also helps to

explain the reduced transmission of reciprocal translocations induced by spermatogonial irradiation (Ford *et al.*, 1969).

In seeking an explanation for the effects of these translocations on spermatogenesis we should probably look at events very early in meiosis, since this seems to be the stage at which germ-cell depletion is first manifest in those most severely affected. The tendency for one breakpoint to be close to the centromere suggested to Cacheiro, Russell and Swartout (1974) that the sterility resulted from position-effect inactivations, while Searle (1974) thought that disturbances to spermatocyte formation might be expected when homologous C-bands are attached to synaptonemal complexes of very different lengths, because this might interfere with attachments to the nuclear membrane. Moses, Russell and Cacheiro (1977) have studied synaptonemal complexes in two male-sterile X-autosome translocations and found a disturbance of X-Y synapsis in one, in which the breakpoint was in the pairing region of the X. One other interesting finding which may throw further light on the nature of the basic disorder is that of Forejt (1974, 1977), that there is an abnormally high frequency of C-band contact between the X chromosome and the translocation configuration in a number of translocations affecting male fertility. However, much more work will be needed before it becomes clear to what extent the wide range of chromosome anomalies affecting meiotic stages of spermatogenesis share a common link in their causation.

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Résumé. Beaucoup de cas de stérilité mâle (mais non femelle) peuvent être rattachés chez la Souris à la présence d'anomalies chromosomiques spécifiques, parmi lesquelles les situations XXX, XYY, les défauts d'association entre X et Y, les hétérozygotes pour les translocations réciproques impliquant les chromosomes X et Y aussi bien que quelques autres translocations où sont en cause seulement les autosomes, les modifications trisomiques tertiaires de ces anomalies et certaines translocations Robertsoniennes multiples. Dans tous ces cas la stérilité résulte d'un trouble de la spermatogenèse qui entraîne l'oligospermie ou l'azoospermie. Les études des translocations réciproques sont d'un intérêt particulier pour élucider les mécanismes impliqués, parce qu'elles offrent une gamme complète d'effets, allant de la production normale de spermatozoïdes à l'arrêt de la spermatogenèse au début de la méiose. Entre ces deux extrêmes, on peut reconnaître des types d'anomalies caractéristiques, mais en général l'arrêt de la spermatogenèse n'est pas total, mais plutôt graduel. Les anomalies de la spermatogenèse sont associables aux caractéristiques particulières des translocations elles-mêmes, particulièrement avec une tendance pour qu'un point de l'échange soit près ou même dans l'hétérochromatine centromérique et l'autre assez loin sur le chromosome en cause. Ceci conduit fréquemment à la formation de chromosomes marqueurs, longs et courts, et à la production de souris à trisomie tertiaire viables qui sont aussi en règle générale des mâles stériles.

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